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## A NEW SPECIES OF BOTRYTIS ON RHIZOMATOUS IRIS

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(WITH PLATES 15 AND 16 AND 1 TEXT FIGURE)

On at least seven occasions during the past ten years, iris rhizomes attacked by a species of *Botrytis*, have been intercepted by inspectors of the U. S. D. A. Plant Quarantine and Control Administration in shipments from France, Germany, England, and Holland. Some of these specimens were submitted to the senior author for examination at the time of interception. Others have been kindly loaned to us by Dr. J. A. Stevenson of the Office of Mycology and Plant Diseases. In addition to these records, the senior author has had affected plants under observation since May 1924 in an iris plantation at Ithaca, N. Y. In June 1927 the junior author found an iris infected by the same fungus in a nursery near Ottawa, Ontario. In January 1931 Dr. Freeman Weiss sent us, from Washington, D. C., some diseased iris rhizomes taken from three shipments grown in the state of Washington. These were covered with the same *Botrytis*. Subsequently, after a visit to the Washington State plantation, Dr. Weiss, in a letter, describes the disease as very destructive, the greater part of the planting of at least two or three acres having been severely affected.

In view of the widespread and serious nature of the disease, it seems desirable to publish a preliminary account, describing and naming the fungus involved. The only published reference to this fungus known to us is a short note by the junior author on its occurrence in Ontario, accompanied by a photograph of a diseased

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rhizome.[1] In the cases thus far observed the fungus has been confined to varieties of the garden iris derived from the species *I. germanica, I. pallida, and I. plicata.*

## PATHOGENY

The names "rhizome rot" and "crown rot," either of which might be applied to the disease caused by this fungus, are used fairly generally for the diseases of rhizomatous iris caused by *Bacillus carotovorus* Jones and *Sclerotium Delphinii* Welch, respectively. The one here described will be referred to as the "*Botrytis* rhizome rot."

Affected plants either fail to develop new leaves in the spring or a few shoots may appear which later turn yellow and finally die by midsummer. On the exposed portions of the rhizomes and at the bases of the leaf sheaths of the previous year's growth, in fact often involving the entire shoot, a dense short felt of dark grey to purplish-brown conidiophores and conidia of the *Botrytis* develop very early in the spring. On the surface of the rhizomes or breaking through the epidermis among the conidiophores and in the soil among the dead roots are agglomerations of characteristically convolute, shiny black sclerotia (PLATE 15, FIG. 1). The plant is easily removed from the soil because of the death and decay of the roots. The rhizomes are shrivelled and partially or completely decayed; the diseased flesh is grey-brown in color, essentially dry and pithy in texture, with distinct rifts in the disintegrating tissues. In partially affected rhizomes, distinct zones of decay may be noted, with a darker colored band sharply delimiting the diseased from the healthy tissue. No disagreeable odor accompanies this decay.

The causal relation of the *Botrytis* to the lesions with which it is constantly associated is hardly to be questioned. The pathogenicity of the fungus has been proven by repeated inoculation of healthy rhizomes in moist chambers in the laboratory. Freshly divided rhizomes of three varieties of iris were inoculated with cultures of six isolates growing on wheat, when planted in the field on November 4, 1931. The following April, all the inoculated plants were dead and covered with sclerotia and conidiophores of

[1] Drayton, F. L. In Report of the Dominion Botanist. Dominion of Canada. Department of Agriculture **1927**: 22–23, fig. 1.

the pathogene. The check plants were perfectly sound. Infection apparently occurs only when the inoculum is introduced into wounds, and is most prompt and extensive at 12°–18° C. At 20°–22° C. invasion is quickly restricted by the formation of a wound periderm about the lesion.

Just when and how invasion occurs in the field remains to be discovered. All the evidence available points to entrance through wounds of one kind or another, with pathogenic activity occurring only during autumn, winter, and early spring. A detailed study of the disease including the life history of the pathogene is under way. This paper deals primarily with the morphology and description of the heretofore unnamed species of *Botrytis* which causes the disease.

## CULTURAL CHARACTERS

Pure cultures of the fungus may be obtained readily on any of the common culture media from plantings of decayed tissue, sclerotia, or spores. Conidiophores, conidia, and the convolute sclerotia are promptly produced in such cultures.

The fungus in its cultural characters is very distinct from any other species of *Botrytis* with which the authors are familiar. The senior author has studied over one thousand isolates of *Botrytis* species from many hosts but has never seen another species markedly resembling this one. Several isolations of *Botrytis* species of the cinerea type having been made from the leaves, and inflorescence of iris both in the United States and Europe, but this one attacking the rhizomes is quite distinct and could scarcely be confused with any of the others.

The sclerotia present the same convoluted agglomerated aspect on culture media as they do on the rhizomes. The conidia also tend to develop a more or less dense felty growth on culture media, similar to that on the host in nature. The optimum temperature for their production in culture is 20° C.

On potato dextrose agar [2] growth is rapid, giving a continuous mat of aërial white mycelium which later becomes buff colored. Conidia are usually produced in tufts or patches over the surface of the media, varying in abundance with conditions of light,

[2] 400 grams potatoes per liter of water, 2 per cent dextrose and 2 per cent agar.

temperature, and humidity. Sclerotia also vary in abundance from single ones 1–3 mm. in diameter up to agglomerations 16–18 mm. across.

Development on oatmeal agar [3] is vigorous and very similar to that on potato dextrose agar.

On Czapek's agar conidiophores cover the entire surface of the media in a dense short felt. Sclerotia are usually numerous but the agglomerations are smaller than on potato dextrose agar.

On nutrient agar, growth is slow, with no aërial mycelium. Neither conidia nor sclerotia have developed in our cultures on this medium. The submerged radiating mycelial mat exhibits an irregular wavy margin.

Abundant production of conidial fructification and sclerotia occur on bean plugs, steamed wheat, and on steamed stems of various succulent plants. (PLATE 16, FIG. 3.)

Appresoria develop in all culture media tested (except nutrient agar) wherever the aërial mycelium comes in contact with the glass sides of the culture vessel. These appresoria are typical of those formed by other species of *Botrytis* and *Sclerotinia*.

Microconidia are produced in great abundance, usually in four to six weeks in old cultures on potato dextrose agar.

## MORPHOLOGY

The mycelium is much branched, hyaline, septate, 4.5–6 $\mu$ in diameter when young; the older hyphae are larger and more closely septate, definitely constricted at the septa, 6–7.5 $\mu$ in diameter, becoming tan colored.

The pale brown conidiophores arise in fascicles from dark, thick-walled, closely septate hyphae near the surface of the substratum (PLATE 16 FIG. 4) or from the sclerotia. They vary in height from 0.82 to 1.12 mm. and in diameter from 9–12 $\mu$ at their bases to 6–7.2 $\mu$ near the apices. Each conidiophore consists of a main axis, toward the apex of which are given off several short branches more or less extensively divaricate. The ultimate hyaline thin walled branchlets of the developing conidiophore are dichotomously forked, the tips swelling to form the "ampullae" [4] upon which numerous spiney sterigmata appear. The tips of

[3] 50 grams rolled oats per liter of water, agar 2 per cent.

[4] The term used by Klebahn in Zeitschrift für Botanik **23**: 251–272. 1930.

the sterigmata rapidly swell to form the young conidia (TEXT FIG. 1). The short sterigmata on which these spores are borne are to be distinguished only before the conidia are full grown. When the spores are mature traces of the sterigmata are no longer to be discovered neither on the conidia nor on the collapsed ampullae.

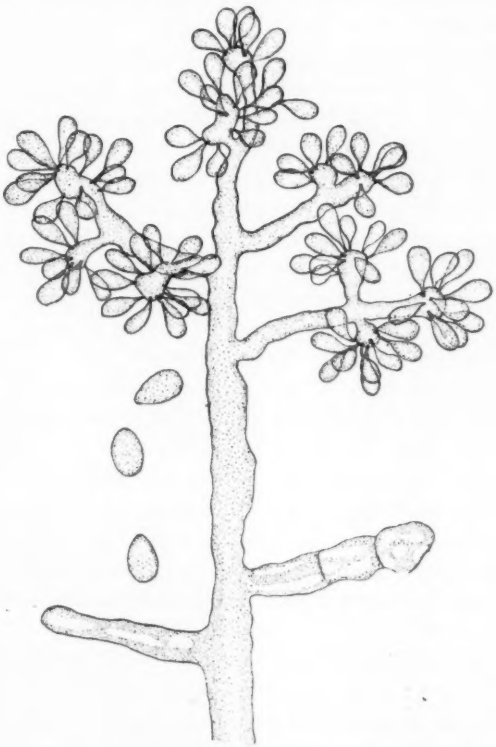

FIG. 1. A young conidiophore, showing the swollen terminal branches (ampullae) on which the conidia are borne. Mature conidia detached.

With maturity of the conidia the branchlets become septate, collapse, and drop away, leaving only the main axis with one or more of the main branches. The positions of the abscissed branches and branchlets are marked by raised circular scars. The final collapse of the ampullae and branchlets appears to be due to

the drainage of their protoplasm into the maturing conidia. The conidia usually drop away singly, but one may frequently see in a mount, clusters of these spores still attached to a shrivelled terminal branchlet which has become detached.[5] Under favorable conditions of food and humidity proliferation of the conidiophores takes place, the new axis arising just laterally of the tip of the old one.

The mature conidia are light brown, ovate to slightly pyriform, unicellular although an occasional abnormal uniseptate individual is encountered. Their size is variable depending apparently on the type of substratum on which they are grown. The following records will illustrate this variability. In each case two hundred conidia mounted in water were measured. Specimen B1036 from freshly collected diseased rhizomes bore conidia having a range of 7–18 × 5.25–12.75 μ, mode 11.0–11.75 × 9.0–9.75 μ and averaging 11.41 × 9.25 μ, compared with which cultures from this same collection grown on potato dextrose agar give conidia with a range of 6.72–16.8 × 5.04–11.76 μ, a mode of 10.08–11.76 × 6.72–8.4 μ, and averaging 11.32 × 7.61 μ; distinctly smaller it will be noted. Conidia from dry herbarium material of this same collection (B1036) gives a range of 6.0–13.5 × 4.75–10.0 μ, a mode of 10.0–10.75 × 8.0–8.75 μ and an average of 9.05 × 7.22 μ, measurements as might be expected distinctly smaller than those of freshly collected living conidia. Conidia of another isolate (B927) growing on potato dextrose agar, Czapek's agar, and bean plugs, give measurements varying slightly from those of B1036 on potato dextrose agar as recorded above.

The sclerotia when mature are shining black, much convoluted to form more or less globose masses (PLATE 15 FIG. 2), which are frequently agglomerated in large clusters on the rotted rhizomes. They present the same aspect in cultures on all media rich in carbohydrates. A single convolute sclerotium may be as large as 18 × 16 mm. although in general they average considerably smaller. When held for a time at low temperature and then

[5] The non-wettability of the conidia and conidiophores of *Botrytis* in water or glycerin makes it almost impossible to obtain satisfactory mounts for critical study in such mounting media. It was discovered that purified mineral oil (Nujol) is a perfect mounting medium for the conidiophore of *Botrytis* and similar fungi.

brought into a temperature of 20°–22° C. the sclerotia promptly germinate producing over their surfaces numerous tufts or fascicles of conidiophores with conidia. The convolute character of the sclerotia distinguishes this *Botrytis* from all the hundreds of forms we have had under observation during the past twenty years. It is because of its peculiar sclerotia that we designate this species as new.

Appresoria are typical of those produced by other *Botrytis* forms of the cinerea type.

The microconidia which have been observed as yet only in pure cultures, undoubtedly occur in nature and presumably function as fertilizing sperms in the production of as yet unobserved apothecial fruit bodies of the *Sclerotinia* type, just as the junior author has already shown to be the function of the microconidia in *Sclerotium Gladioli* Massey.[6] They are globose, hyaline, 2.5–4.5 $\mu$ in diameter, produced on typical fasciculate conidiophores arising from single cells in the hyphae about the bases of conidiophores or on the sclerotia (PLATE 16 FIG. 5). They are produced in the greatest abundance in this species appearing as viscous turbid droplets varying in size from a pin point to a millimeter in diameter.

## TECHNICAL DESCRIPTION

**Botrytis convoluta** sp. nov.

Mycelium profusely branching, septate, hyaline, becoming tan colored with age at surface of the substrate. Sclerotia shining black, convolute, agglomerated, up to 18 × 16 mm. in size. Conidiophores brown, erect, fasciculate, branched at the apex, about 1 mm. tall, 9–12 $\mu$ in diameter at the base, tapering toward the apex, arising from large dark thick-walled cells in the mycelium or from medullary cells just beneath the rind of the sclerotia. Conidia light brown, one-celled, smooth, ovate to slightly pyriform, borne in dense clusters on sterigmata produced from the swollen ampullae of the ultimate branchlets of the conidiophores; size variable, living spores from diseased rhizomes range from 7–18 × 5.25–12.75 $\mu$, mode 11.0–11.75 × 9.0–9.75 $\mu$, average 11.41 × 9.25 $\mu$; somewhat smaller when produced on culture media.

[6] Drayton, F. L., Mycologia **24**: 345–348. 1932.

Microconidia globose 2.5–4.5 $\mu$ in diameter, produced successively from the tips of obclavate conidiophores, arising in densely branched fascicles from single large globose or obovate hyphal cells of the mycelium or from the sclerotial medulla.

Sclerotiis atro-nitentibus convolutis agglutinatis, usque ad 18 × 16 mm.; conidiophoris brunneis, erectis, fasciculatis apice ramosis, circa 1 mm. altis, e cellulis callosis mycelii aut e sclerotiis orientibus; conidiis pallide brunneis, ovatis vel pyriformibus, 7–18 $\mu$ longis, 5.25–12.75 $\mu$ diam.; microconidiis globosis 2.5–4.5 $\mu$ diam.

Parasitic on rhizomes of species of rhizomatous Iris. To be found in early spring (March and April). Known from Germany, France, Holland, England, United States and Canada. Type specimen deposited in Plant Pathological Herbarium Cornell University, Ithaca, N. Y. *No. 12615*.

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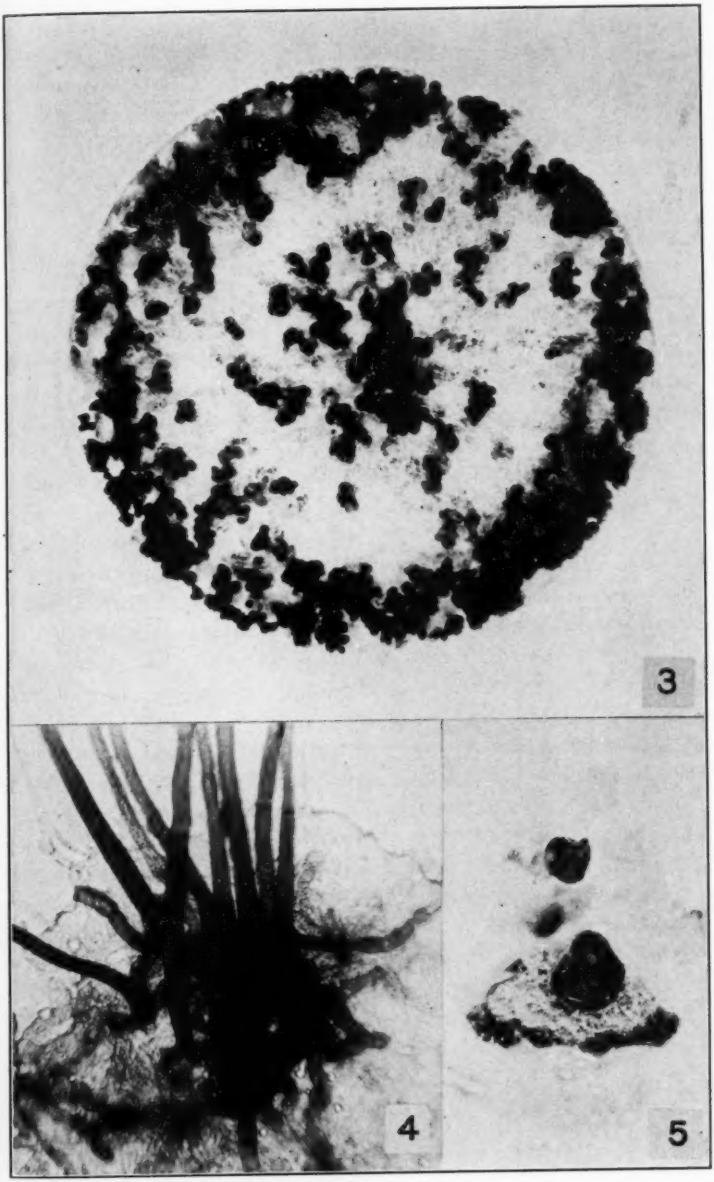
## EXPLANATION OF PLATES

Plate 15, Fig. 1. A diseased iris plant. The leaves are decayed, the rhizome shrivelled, the roots decayed, masses of sclerotia and conidiophores arising from the tissues; Fig. 2. Sclerotial agglomerations enlarged 5×.

Plate 16, Fig. 3. A petri dish culture on sterilized wheat; Fig. 4, Conidiophores arising from large hyphal cells; Fig. 5, Sclerotia with attached microconidial sporodochia.




BOTRYTIS CONVOLUTA

# THREE NEW SPECIES OF MYTILIDION IN THE PROPOSED SUBGENUS, LOPHIOPSIS [1]

M. L. LOHMAN

(WITH PLATE 17 AND 1 TEXT FIGURE)

The species of hysteriaceous fungi described in this paper as new are worthy of special note; not so much in that their slender asci and sinuous spores segregate them from known species of *Mytilidion* to a degree which, in the opinion of the writer, is properly emphasized in the establishment of the new subgenus *Lophiopsis*, but rather in that they connect more closely the heretofore somewhat isolated genus *Lophium*, with the genera of those species which have more truly hysteriform fructifications. As transitional forms their diagnostic features are those which might have been assumed; namely, (1) thin-walled, carbonaceous, conchiform hysterothecia typical of the species of both *Lophium* and *Mytilidion*, (2) subcylindrical or cylindrical asci as known in species of *Lophium*, and (3) colored ascospores (of the form commonly termed scolecosporous) which are more elongate than those known for *Mytilidion* but which, as in species of *Mytilidion*, do not exceed in their length half that of the ascus, presenting, therefore, a more or less biseriate arrangement in the ascus as opposed to the fasciculate grouping of the spores in the linosporous genus, *Lophium*. Thus, it is upon the basis of the length of the ascospore, relative to that of the ascus, that the species are referred to *Mytilidion* rather than to *Lophium*, and upon the basis of the ratio of length of spore to breadth of spore that the subgenus *Lophiopsis* is proposed to receive them. Two of the three species are now being cultivated. The color, rate of growth, and nature of their mycelia (as compared with the mycelial characteristics of a number of species of *Mytilidion* and of *Lophium mytilinum* (Pers.) Fries and *L. dolabriforme* Wallr.) support this disposition of the three species.

[1] Contribution No. 109 from the Laboratories of Cryptogamic Botany Harvard University.

A study of the following emendation will reveal that the reference of these species to the genus *Mytilidion*, established by Duby (**2**) in 1861, necessitates no grave alteration of our concept of the genus other than extending somewhat the limits of the characteristics of the ascospore.

MYTILIDION Duby (*l. c., sub nom. Mytilinidion*), emended

Hysterothecia superficial, prosenchymatous, fragile-carbonaceous, sessile and erect with the lateral walls more or less connivent and extended vertically. Asci 8-spored, clavate to subcylindric. Ascospores colored, elliptic-oblong to fusiform or (in the subgenus *Lophiopsis*) elongate-fusiform to slender-clavate, with three or more septa.

MYTILIDION Duby, emend.—Hysterothecia superficialia, prosenchymatica, fragilia carbonacea, sessilia, verticalia, labiis plerumque acutis arte conniventibus. Asci octospori, clavati vel subcylindrici. Sporidia flavescentia, aut elliptico-oblonga aut (in subg. *Lophiopsis*) elongato clavulata, 3- — pluriseptata.

## SUBGENUS I. EU-MYTILIDION

*Spores clear yellow-brown to fuscous, with a ratio of length to breadth of approximately 10 : 1 or less.*

In this subgenus (which is the genus *Mytilidion* of most authors) there is correlated with the ratio of measurements—a factor of practical taxonomic value—the phenomenon of polarity in germination, *i.e.*, the terminal cells of the spore germinate first, and often only these cells germinate. This additional factor is authenticated by the writer's studies (**3**) concerning germinating spores in the species, *M. tortile* (Schw.) Sacc. and *M. laeviusculum* (Karst.) Sacc., in American specimens referred with considerable doubt to *M. decipiens* (Karst.) Sacc., and in a species being described elsewhere (**4**) as new.

## SPECIES KNOWN TO OCCUR IN THE CENTRAL AND EASTERN STATES

Although extraneous to this paper in view of its title, the following notes on species known to occur in the central and eastern states are given, since, in concerning species probably not uncommon in this country on the bark or wood of conifers, they may be helpful to those who attempt a systematic study of this genus.

*Mytilidion tortile* (Schw.) Sacc. is a well-defined species in this country, as Bisby (**1**) has concluded. In view of the discrepancies in the various descriptions under this name, the writer is presenting elsewhere (**4**) a rather detailed diagnosis of the species, based upon his observations of the fungus in culture and upon his study of *Hysterium tortile* Schw. (*2065* in the herbarium of Schweinitz at Philadelphia).

*Mytilidion Karstenii* Sacc. (as originally described by Karsten) with spores 38–49 × 4–4.5 μ, only slightly tapered to the truncate upper end, has been collected in New England from the bark of *Pinus*, while *M. laeviusculum* (Karst.) Sacc. (as described by Karsten; *cf.* **4**) has been collected in Michigan from unexposed surfaces of the wood of *Larix*.

*Hysterium Thujarum* Cooke & Peck, for which Bisby (**1**) has described reasonably authentic material, is a good species and one which, in the opinion of the writer (see **4**), is more properly considered as of the genus *Mytilidion*. The species appears to be common in northern Michigan and Wisconsin, at least where cut-over stands of *Thuja* are encountered. Collections which have come from this area indicate that the species is as variable in its morphology, and thus as perturbing to the student, as is either *Hysterium insidens* Schw. or *Hysterographium Mori* (Schw.) Rehm.

For *Mytilidion Karstenii* and *M. laeviusculum*, species mentioned above, and for *M. decipiens* (Karst.) Sacc. and *M. fusisporum* (Cooke) Sacc., species that have been reported as occurring in these regions, no critical notes based upon truly authentic material are available.

## SUBGENUS II. LOPHIOPSIS

*Spores yellowish to yellow-brown, with a ratio of length to breadth of approximately 20 : 1.*

In this subgenus there is correlated with the ratio of measurements of the ascospore a condition of non-polarity in germination, *i.e.*, any cell of the spore may be the first to germinate (as in *Lophium mytilinum* (Pers.) Fries, *cf.* **3**). It is proposed for an adequate disposition of the three following species.

**Mytilidion scolecosporum** Lohman, sp. nov. FIGURE 1, *C*. PLATE 17, *A*

Syn: *Septonema toruloideum* Cooke & Ellis, Grevillea **7**: 6. 1878 [2]

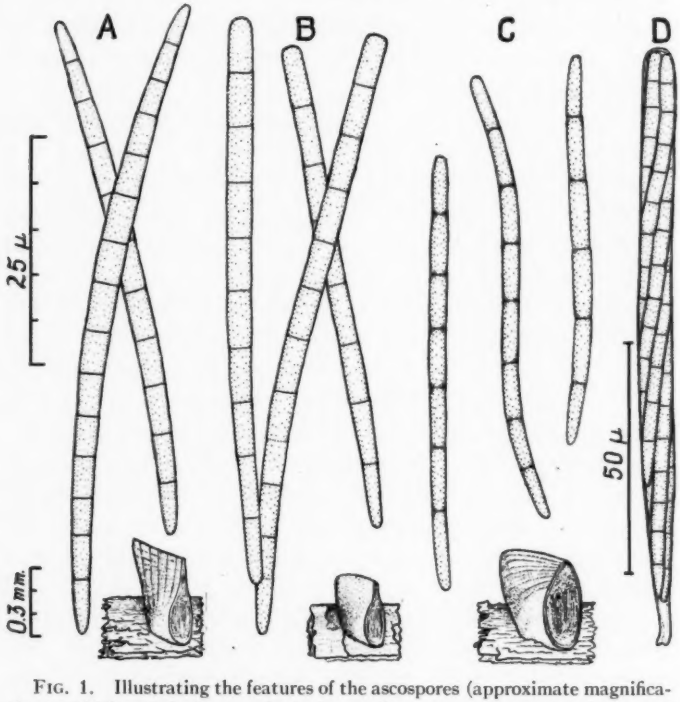



FIG. 1. Illustrating the features of the ascospores (approximate magnification × 1335) and the hysterothecia (approximate magnification of the halved fructifications, sketched in perspective, × 33) for *Mytilidion australe* (*A*), *M. parvulum* (*B*), and *M. scolecosporum* (*C*); also, the form of the asci and arrangement of the spores, as shown in *D* for *M. parvulum* (approximate magnification ×665).

Hysterothecia conchiform but not acutely keeled, densely gregarious, 0.4–0.8 (1) × 0.2–0.3 mm. (0.2–0.4 mm. in height), dull black and longitudinally striate, occasionally three-radiate and erect, or in pairs and horizontally disposed, superficial from the beginning on an effused black crust made more prominent in places by the minute, punctiform centers of conidial sporulation; walls prosenchymatous, thin, carbonaceous and fragile; asci

[2] See next Mycologia.

subcylindric, 100–130 × 4–5.5 μ; paraphyses delicate, hyaline, septate, sparingly branched and interwoven above; spores 40–50 × 2–2.5 μ, subvermiform, occasionally bent or subsigmoid, yellowish to clear brown, subspirally biseriate, 5- to 7-septate and slightly constricted at the septa; conidia elliptic-oblong, tapered apically, deep fuscous throughout or with one or two of the apical cells paler, 3-to 5-septate, 14–18 (24) × 4.5–5 (6) μ, deeply constricted, arranged in erect or variously decumbent, simple or sparingly branched, easily broken chains 75 to 200 μ in length; pycnidia unknown.

On wood of much weathered stump of *Pinus Strobus* L., Green Bay, Wis., Sept. 7, 1930. Collected by A. H. Smith. Type specimen in the Farlow Herbarium at Harvard University and material from the type collection in the University of Michigan Herbarium.

**Mytilidion scolecosporum** Lohman, sp. nov.—hysterotheciis conchiformibus haud quaquam acute cristatis, interdum triradiatim partitis, 0.4–0.8 (1) mm. longis, 0.2–0.3 mm. latis, 0.2–0.4 mm. altis, dense gregariis, nigris, longitudinaliter striatis, prosenchymaticis, fragilibus carbonaceis, superficialibus, plerumque ad crustam atrofuscam status hyphomyceti dispositis; ascis subcylindratis, octosporis, 100–130 × 4–5.5 μ; paraphysibus filiformibus, tenellis, superne ramosis et intertextis; sporidiis 40–50 × 2–2.5 μ, fortiter elongatis, tenuibus, leniter flexis, flavescentibus, subspiraliter distichis, 5- –7-septatis, ad septa paulo constrictis; conidiis concatenatis, aut omnino fuscis aut luteolioribus ad apicem, (3) 5-septatis, constrictis, 14–18 (24) × 4.5–5 (6) μ, catenis inordinatis, 75–200 μ longis, fragilibus, simplicibus ramosisve.

This species, distinct in its subvermiform spores, shows its relationship to the species of the preceding section through *M. Karstenii* Sacc., *M. rhenanum* Fuckel, and *M. Thujae* Feltig.

**Mytilidion parvulum** Lohman, sp. nov. FIGURE 1, *B*, *D*. PLATE 17, *B*, *C*

Hysterothecia conchiform and acutely keeled, superficial, black and shining, 0.3–0.5 × 0.15—0.18 mm. (0.2–0.3 mm. in height), arranged in loose but widespread aggregations which blacken the substratum; walls prosenchymatous, thin, carbonaceous and fragile; asci subcylindric, 8-spored, 120–130 (135) ×6–7.5 μ; paraphyses sparse, delicate, hyaline, septate, sparingly branched and interwoven above; spores (48) 54–62 (65) × 2.7–3 μ, slender clavate with the upper end broadly obtuse and the lower pointed, usually slightly bent in the lower half, yellowish brown, subspirally biseriate, 7- to 9-septate (or becoming 11-septate by less distinct walls through several of the cells) and unconstricted.

On bark and wood of old stump (*Pinus*), Sharon, Mass., Dec. 5, 1908. Collected by A. P. D. Piquet. Type specimen in the Farlow Herbarium at Harvard University.[3]

**Mytilidion parvulum** Lohman, sp. nov.—hysterotheciis conchiformibus, superficialibus, atribus nitidisque, 0.3–0.5 mm. longis, 0.15–0.18 mm. latis, 0.2–0.3 mm. altis, laxe gregariis, prosenchymaticis, fragilibus carbonaceis; ascis subcylindratis, octosporis, 120–130 (135) × 6–7.5 μ; paraphysibus sparsis, filiformibus, tenellis, superne ramosis et intertextis; sporidiis (48) 54–62 (65) × 2.7–3 μ, valde elongatis, clavulatis, plerumque in deorsam semipartem leniter flexis, flavidis luteofuscisve, subspiraliter distichis, inconstrictis, aut 7- –9-septatis aut ullo loculo etiam indistincte partito, quo 11-septatis.

Although its fructifications are small, as the specific name implies, the species has longer spores than does either *M. scolecosporum* or *M. Karstenii*. Hence, it approaches *Lophium mytilinum* more closely than does either of the two species just mentioned.

**Mytilidion australe** Lohman, sp. nov. FIGURE 1, *A*. PLATE 17, *D–F*

Hysterothecia vertically appressed with fan-shaped crests, densely aggregated in small scattered clusters, 0.4–0.6 (0.8) × 0.15–0.2 mm. (0.3–0.4 mm. in height), vertically and longitudinally striate, black and shining; walls prosenchymatous, thin, carbonaceous and fragile; asci subcylindric, 8-spored, 125–150 × 8–9 μ; paraphyses sparse, delicate, hyaline, septate, branched and interwoven above; spores (54) 58–70 (75) × 3–4 μ, elongate, tapered equally toward each end, slightly curved to sublunate, yellowish, subspirally biseriate, (10) 11- to 14-septate and unconstricted.

On much decayed wood of *Pinus*, Baton Rouge, La., Dec. 27, 1931. Collected by A. H. Smith. Type specimen in the Farlow Herbarium at Harvard University and material from the type collection in the University of Michigan Herbarium.

**Mytilidion australe** Lohman, sp. nov.—hysterotheciis conchiformibus, pertenuibus, quasi flabelliformibus, densis in greges parvos sparsos, 0.4–0.6 (0.8) mm. longis, 0.15–0.2 mm. latis, 0.3–0.4 mm. altis, verticaliter ac longitudinaliter striatis, atribus, nitidis, prosenchymaticis, fragilibus carbonaceis; ascis subcylindratis, octosporis, 125–150 × 8–9 μ; paraphysibus sparsis, filiformibus, tenellulis, superne ramosis et intertextis; sporidiis (54) 58–70 (75) × 3–4 μ, elongatis, leniter flexis sublunatisve, flavescentibus, subspiraliter distichis, (10) 11- quoad 14-septatisve, inconstrictis.

[3] Dr. Farlow had studied the specimens of this collection and had noted that they represented an undescribed species of *Mytilidion*.

Since there is no black fungous layer present, the rather scattered, slender fructifications on the weathered wood are scarcely noticeable to the unaided eye. The long, slightly curved spores with many septa could not be confused with those of any known species of the genus.

## CONCLUDING REMARKS

Although at the present time each of the three foregoing species is known to occur only on *Pinus* and at the respective station indicated in its description, the species undoubtedly occur elsewhere and possibly on conifers other than *Pinus*. The habits of *Lophium mytilinum* and certain species of *Mytilidion* lead one to believe that *M. scolecosporum* and *M. australe* are likely to be encountered on bark as well as on decayed wood. However, since certain *Mytilidion*-like species show a specificity to a single generic coniferous substrate, special effort should be made to identify the wood or bark in recording the occurrence of these fungi.

Differing from *Lophium mytilinum* in the ratio of length to breadth of the ascospore and in the arrangement of the spores in the ascus although resembling that species in the method of germination of the ascospore, these species are referred to the new subgenus, *Lophiopsis*, proposed as a segregation of *Mytilidion*. Intermediate with respect to certain diagnostic features between *Lophium* and *Mytilidion* as conceived by earlier students of the Hysteriaceae, these species constitute a significant step in advance in our knowledge of the interspecific relationships of those hysteriaceous fungi which appear to be more or less confined to coniferous substrata.

Two of the three species, namely, *Mytilidion scolecosporum* and *M. australe*, are being studied in culture in connection with further investigations into the life histories of members of the Hysteriaceae,—studies which are being pursued by the writer as a National Research Fellow under the sponsorship of Prof. William H. Weston, Jr.

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## EXPLANATION OF PLATE 17

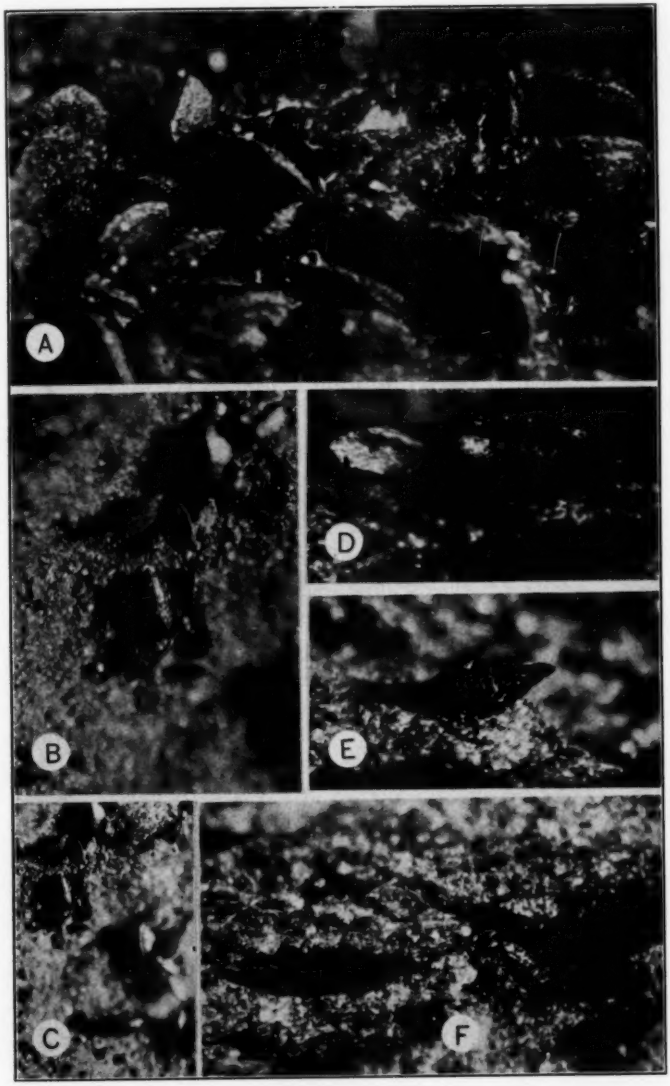
Hysterothecial habits of three new species of *Mytilidion*

The photographs illustrate portions of the type specimens for these species and as reproduced they represent an approximate magnification of 30 ×, with the exception of *C*, which is × 15. (One not especially familiar with *conchiform* fructifications should study in connection with the photographs the rather schematic drawings of halved fructifications shown in the text figure.)

*A*. *Mytilidion scolecosporum* on weathered wood of *Pinus*. The illustration includes about twenty fructifications surrounded and overlain in part by the effused conidial stage (*Septonema toruloideum* Cooke & Ellis). Centers of conidial sporulation are most evident near the left margin.

*B*, *C*. *Mytilidion parvulum* on bark from an old stump of *Pinus*. *B*, a group of seven fructifications, is an enlargement of the upper portion of *C*.

*D*.–*F*. *Mytilidion australe* on decayed wood of *Pinus*. *D*, a small group of fructifications as viewed from the side; *E*, a single fructification as observed obliquely; *F*, a single fructification (in central, left portion) as seen from above and two fructifications (in right portion) as viewed laterally.




MYTILIDION

# IDENTIFICATION OF DIAPORTHE UMBRINA ON ROSE FROM ENGLAND [1]

ANNA E. JENKINS AND R. P. WHITE

(WITH PLATES 18 AND 19)

## INTRODUCTION

In November, 1931, a cankered rose-stem specimen from England was sent to the senior author by G. H. Pethybridge, Mycologist of the Ministry of Agriculture and Fisheries, Harpenden, Hertfordshire, to learn whether the fungus present might not be *Diaporthe umbrina* Jenkins. The specimen was collected at Cheltenham, Gloucestershire, in October, 1931 by L. Ogilvie, Advisory Mycologist at the Agricultural and Horticultural Research Station, Bristol. The fungus on this specimen is unquestionably what is called *Diaporthe umbrina* in the United States. It seems likely that the brown canker rose disease caused by this organism is not as prevalent in England as in the United States, or it would have been recognized earlier by its generally definite and conspicuous symptoms.

Identified as *Diaporthe umbrina*, this fungus, causing brown canker of roses, has not heretofore been reported from England or elsewhere than in the United States, although attempts have previously been made on the part of the senior author to learn whether it was of wider distribution. Together with certain other diseases, actual search for this disease was made in various rose plantings in continental Europe and in England and Scotland, in 1929–1930, by Cynthia Westcott, then Heckscher Research Assistant, Department of Plant Pathology, Cornell University, Ithaca, N. Y., and in 1930, in similar regions by the junior author, and in England and Scotland by the senior, who examined roses both growing wild and cultivated. Westcott saw what appeared to be brown canker in a certain rose garden in

[1] Joint contribution from the Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture and the Department of Plant Pathology, New Jersey Agricultural Experiment Station.

England, but otherwise no evidence of the occurrence of the disease resulted from this rather limited survey.

Since the identification of the Cheltenham fungus as *Diaporthe umbrina* constitutes the first knowledge of the occurrence in Europe of this important rose pathogene, the studies made in connection with the identification are here reported. This seems particularly advisable since they afford some additional data pertaining to the morphology, cultural characteristics and life history of the organism. As thus recorded these may be of further assistance in its recognition.

The appearance of the cankered stem was typical for brown canker, although from this rather meagre material (PLATE 18, *A* and *B*) positive diagnosis could not be made. The *Diaporthe* was present mostly in its perfect stage. Comparison of this stage (PLATE 19, *A*) with that of *Diaporthe umbrina* from Arlington Experiment Farm, Rosslyn, Va., showed these two stages to agree. It is known, however, that there is a *Diaporthe* on rose, undetermined specifically,[2] that closely resembles *D. umbrina* in its perfect stage, but differs from it in having a typical *Phomopsis* imperfect stage as well as in its cultural characteristics.

Under the circumstances, cultural and other studies of the Cheltenham fungus were made to be certain of its agreement with *Diaporthe umbrina* from the United States. PLATE 18, *E*, shows a 3-months-old, original isolation of the Cheltenham fungus growing on a potato-dextrose agar medium test-tube slant. The color of the conidial masses were here "cinnamon buff"[3] and that of the hyphal growth, "olive brown." From this isolation the fungus was inoculated on blooms and flower stalks of the hybrid tea rose, Lady Margaret Stewart, known to be highly susceptible to *D. umbrina*. In 3 days characteristic brown canker symptoms were produced on the blooms and in 12 days on the stalks, as shown in PLATE 18, *C* and *D*. The imperfect stage of the fungus was visible on the petals, as minute brownish

[2] Diseases of fruit and nut crops in the United States. United States Department of Agriculture Plant Disease Reporter Supplement 39, May 1, 1925, p. 360.

[3] Color readings in this account are based on Ridgway, R. Color standards and color nomenclature, 43 p., illus. Washington, D. C., and most of them were made by J. Marion Shull.

to blackish dots (PLATE 18, *D*). This is entirely characteristic of this stage of *D. umbrina*, which often grows on rose petals in the United States. The re-isolated organism was in agreement with the Cheltenham strain.

Culturally, the Cheltenham fungus was compared with two different isolations of *Diaporthe umbrina* from Arlington Experiment Farm, one of 1923 and the other of 1931, but both of essentially the same appearance. Such cultures on test-tube slants of agar media, potato-dextrose (PLATE 18, *F* and *G*) and corn-meal (PLATE 18, *H* and *I*), and on corn-meal agar media in Petri dishes (PLATE 19, *B* and *D*) are here represented. PLATE 18, *F* and *H*, and PLATE 19, *B*, represent the strain from England; PLATE 18, *G* and *I*, and PLATE 19, *D*, that from the United States, isolated in 1931. As illustrated, the two fungi are in agreement culturally, although they do show slight strain differences. These differences are not uncommon among different isolations of *D. umbrina* from the United States. Perhaps the most notable difference between the English and American cultures here compared is that, in mass, conidia in the culture from England usually differ in color from those of the American cultures. For example, at the time they were photographed, the conidial masses in the Cheltenham cultures shown in PLATE 18, *F*, were "deep olive buff" while those of the corresponding American culture shown in PLATE 18, *G*, ranged from "cream buff" to "chamois." In another instance, wherein the Cheltenham strain and the American isolation of 1923 were compared on oatmeal agar test-tube slants, the conidial masses in the former strain were "antimony yellow" to "yellow ochre" and those in the latter, "chamois." Grown on sterilized corn-meal media in Erlenmeyer flasks the Cheltenham culture was again characteristic of *Diaporthe umbrina* as known in the United States, although the coloration of its spore masses was somewhat different from that of the American strain compared with it.

Conidia, conidiophores, and hyphae of the Cheltenham fungus were in agreement with those of *Diaporthe umbrina*. Comparisons of conidia and conidiophores were made mostly on the basis of this stage as produced in culture and as developed on rose petals. Those of the hyphae were based largely on the hyphal

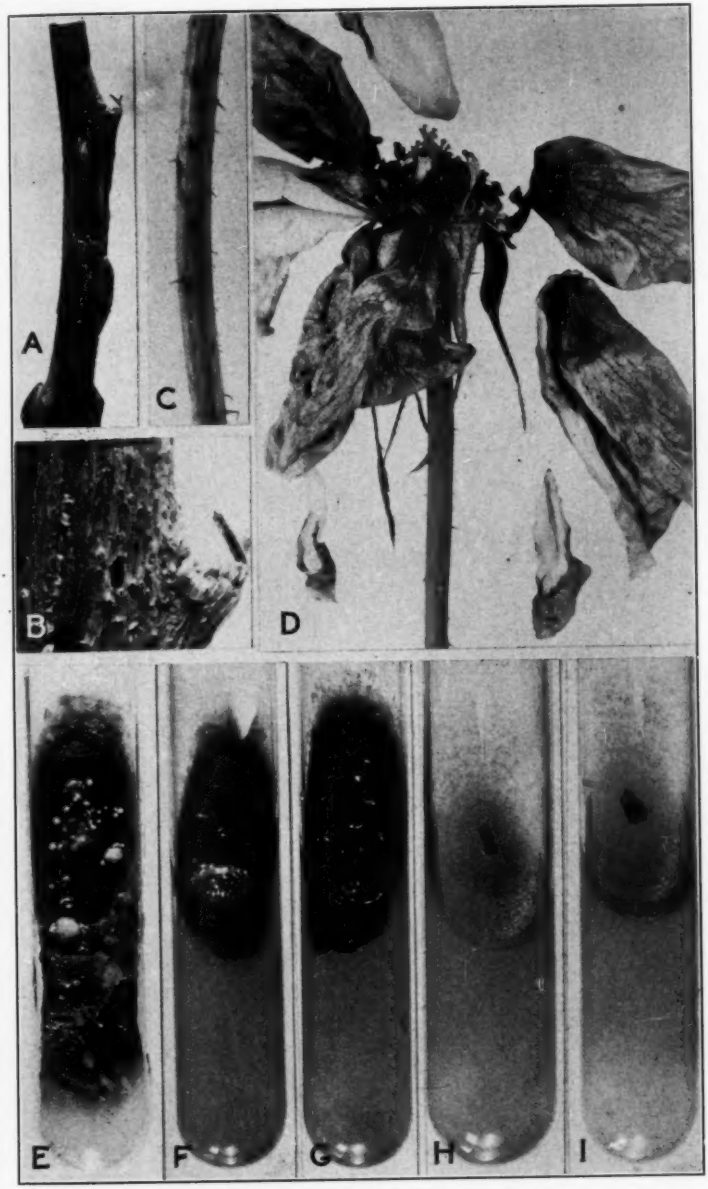
development at the margins of the cultures illustrated in PLATE 19, *B* and *D* (PLATE 19, *C* and *E*).

The data here presented show that, although there are slight cultural differences, the Cheltenham fungus, is to be considered identical with *Diaporthe umbrina*, previously reported only from the United States.

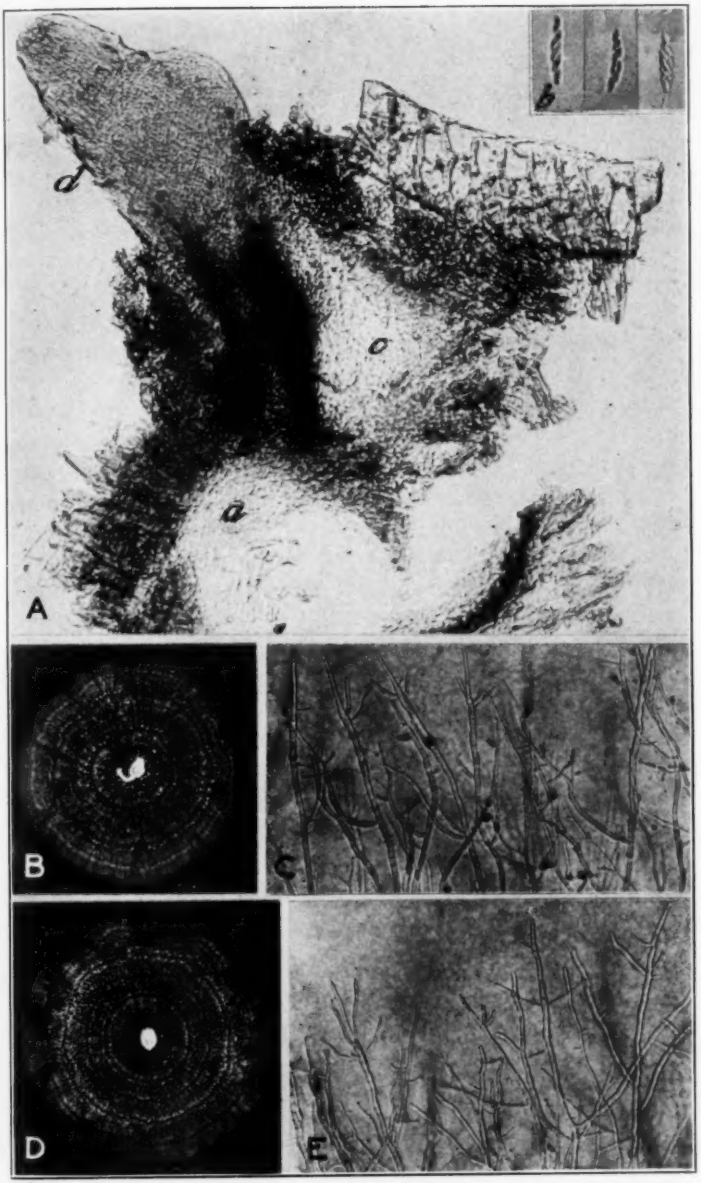
## EXPLANATION OF PLATES

Plate 18. *Diaporthe umbrina*. *A*, specimen sent by G. H. Pethybridge, this having been collected at Cheltenham, Gloucestershire, England, October, 1931, by L. Ogilvie (× 1). *B*, enlargement of *A*, to show perfect stage fructifications of the fungus (× 4). *C* and *D*, rose leaf stalk (*C*) and bloom (*D*) inoculated with culture isolated from Cheltenham fungus, pycnidial stage showing as small dots on petals (*C*, × 2; *D*, × 1). *E*, 3-months-old slant culture on potato-dextrose agar medium, this being the original isolation from the Cheltenham strain (× 1). *F–I*, comparisons of the Cheltenham strain (*F* and *H*) with one of the American strains (*G* and *I*) on 10-day-old slant cultures of potato-dextrose (*F* and *G*), and corn-meal agar media (*H* and *I*) (× 1). Illustrations by M. L. F. Foubert.

Plate 19. *Diaporthe umbrina*. *A*, perithecium from Cheltenham specimen; *a* and *b*, asci; *c*, imperfect stage of fungus at side of perithecium; *d*, beak of perithecium protruding beyond surface of stem (× 200). *B*, week-old, corn-meal agar Petri dish culture of Cheltenham strain (*B*), grown in parallel with a strain from the United States (*D*) (× 1). *C*, hyphae at margin of *B*; *E*, at margin of *D* (× 120). Illustrations by M. L. F. Foubert.




DIAPORTHE UMBRINA




DIAPORTHE UMBRINA

# A NEW PARASITIC PYTHIUM

WILLY HÖHNK

(WITH PLATE 20 AND 11 TEXT FIGURES)

This fungus was obtained from a soil sample [1] in association with *Allomyces*. Its more rapid and vigorous growth hindered and at times completely suffocated the *Allomyces*. In the ice-box at a temperature of about 3° C., *Allomyces* grew slowly but at a fair rate of speed on agar [2] cultures while the *Pythium* grew very slowly or not at all. However, if the petri-dish cultures were kept at room temperature the *Pythium* would grow so rapidly as to smother the *Allomyces*. Because of this variation in growth it was possible to secure pure *Allomyces* at the lower temperatures and pure *Pythium* at the higher temperatures. The pure cultures of the *Pythium* were obtained by cutting out a small portion of agar at the edge of the colony grown under room temperature and transferring the piece to sterile water. After further growth had been established a single sporangium was carried over to a petri-dish containing sterile agar. After 24 hours it would be possible to make further transfers from this growth.

Ant larvae and various seeds were used as a supply of food for the fungus. All were attacked by *Pythium epigynum*. However on ant larvae the hyphae grew longer by about 1–1.5 cm. and very early formed reproductive organs. On hemp seed the

[1] The soil-sample was taken from the surface at the waterline of a pond in a meadow near Milton, Wisc. No plants were present; the grass-cover began at a distance of about 3 m.

[2] The same agar as used for Saprolegniaceae. Its constitution: Within 1 L. distilled water.

8–10 g. agar
5 g. Carragen or if not available 5 g. agar more
0.5 g. Dextrose
0.05 g. Citric acid
0.0005 g. $KH_2PO_4$
0.000025 g. $NH_4NO_3$
0.000025 g. $(NH_4)_2SO_4$
0.000025 g. $MgSO_4$
} stock solution

hyphae were short and so closely interwoven that observation was difficult, consequently ant larvae have been used in carrying out these studies.

The temperature relations of the fungus are shown in the following graphs (FIG. 1, *a* and FIG. 1, *b*).

Into a petri-dish 10 cc. of nutrient-agar were placed. Each of these were then inoculated with 9–12 resting-sporangia of an eight week old water culture. Two dishes were then exposed at the same time, to each degree of temperature, and this was

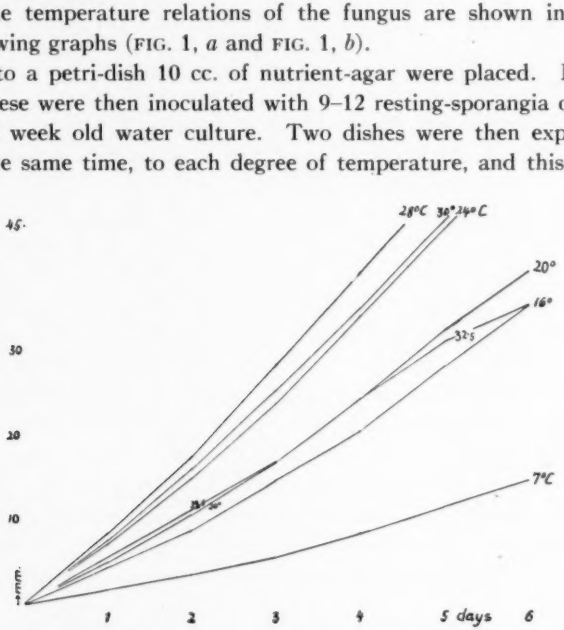



FIG. 1*a*. , Length-growth during 6 days at different temperatures.

repeated twice or, if necessary, three times. The cultures were checked for contaminations and any showing impurity discarded and replaced by fresh cultures. The growth was generally circular and those which were not circular were discarded. They were measured daily in two diameters at right angles. Then the average was taken of all the 4 (or 6) cultures which had been grown for each degree of temperature. The average-diameters were divided by 2 in order to get the length of the radia and these are shown on graph 1*a* and 1*b*. Temperatures were checked daily. The variations were within 1.5° C.

The sporangia exposed to a temperature of 3.5° C. grew to about 300 $\mu$ within a day or two and then stopped. Curves at

7°, 16°, 20°, 24°, 28° and 30° C. are nearly straight (FIG. 1*a*). At all of these temperatures the daily growth equalled, and usually slightly exceeded the first day-rate of growth.—The optimum temperature is close to 28° C. At 30° C. and 24° C. the rates of growth are alike, as they are also at 20° C. and 32.5° C. (FIG. 1*a*), but between the latter two temperatures the lines cross. This point was checked three times with the same result. After three or four days the day-rates at 32.5° C. decreased and the mycelium failed to grow to the edge of the dish. The same occurred at a temperature of 3.5° C., but the duration of growth was only about one day. The same happened at a temperature of 36° C. But at 40° C. no germination or growth occurred and these cultures exposed for 4 or 5 days to a favorable temperature of 28° C. or lower remained sterile. The sharp descent of the curves between 28° C. and 35° C. is remarkable.

## MYCELIUM

Recently infected ant larvae were surrounded by a weft of hyphae at the end of 12–14 hours and the first sporangia were apparent at 24 hours. The main hyphae are branched and of a fairly uniform diameter, about 6 $\mu$. As they elongate the basal portion thickens somewhat, often reaching a diameter of 8 $\mu$. These main hyphae and their oldest broad branches dominate in the turf formed around the ant larva. A culture, left undisturbed for a week, shows the same strict radial growth as in many of the Saprolegniaceae, having thick hyphae. A characteristic type of growth is shown in figure 2. The side branches near the base are the largest and branches are more delicate as one approaches the tip of the main hypha. The same is true of the branches as well. Oftentimes the portion of a hyphae below an intercalary zoösporangium is thicker than the upper portion. This can be repeatedly found and is a result of the mode of formation as will be explained later. The expression "gradually getting thinner" on page 505 in the table of comparison has reference to this peculiarity.—The tips of hyphae are greatly attenuated. The number of lateral branches in a water culture are dependent upon the food available and not upon the amount of water present. If a fresh ant larva is placed close to one

already infected, the branching becomes very profuse and at the same time very irregular.

Within old hyphae (in a medium poor in food and after 3 days), irregular formation of cross walls may be found and these are more frequent in the thin side branches than in the heavy main hyphae.

## SPORANGIA AND GEMMAE

The first formed zoösporangia are spherical or slightly oval in shape, usually intercalary, seldom terminal. The spherical zoösporangia average ± 29 μ (21–34 μ), the oval sporangia aver-

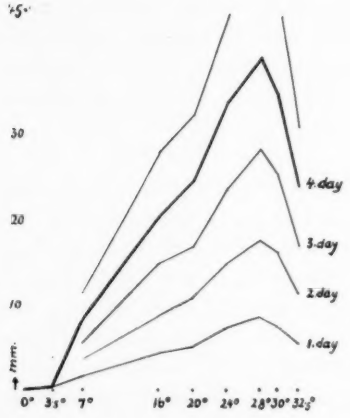



FIG. 1*b*. The curves show the daily growths at different temperatures.

age 19–24 μ: 22–29 μ. Some immediately form zoöspores, while most become resting-sporangia. Those which function immediately push out a long tube (about two times the sporangium diameter), frequently 12 μ in diameter as shown in figure 3. With the development of the tube, a vacuole makes its appearance within the protoplast and increases in size as the protoplast enters the tube and moves into the developing vesicle. The formation of zoöspores and their movement soon brings about a rupture of the vesicle.

Each zoöspore has two lateral cilia and a definite contractile vacuole. Under high magnification the operation of this vacuole

can be readily observed. The time between contraction and expansion becomes longer as the zoöspore matures. The swarm-period of the zoöspores varies from 10–25 minutes. At the end of the swarm-period the zoöspore becomes spherical; cilia and vacuole disappear and a surrounding membrane is soon formed. If fresh water is now added a second swarm period is noted after five hours. The protoplast leaves the membrane as a zoöspore of the same size and shape, with two cilia and a vacuole. Such a spore again undergoes a resting-period before germination.

As said, only a few of the first sporangia undergo sporulation at once. The others become resting sporangia. The same situation holds with reference to the later formed sporangia, only here still fewer of the sporangia germinate at once. The percentage of those which germinate immediately may be influenced by the addition of fresh water to the culture within 4–6 hours. These peculiarities indicate that the terms "primary" and "secondary" sporangia are unfortunate, even misleading. These terms have only descriptive value in describing members of the Prolifera-group of the Pythiaceae and certain genera of the Saprolegniaceae. Here are applied zoösporangia or "sporangia" and "resting sporangia" in order to indicate the possibilities of germination or sporulation later if influenced by increasing age or changes in environment.

A terminal sporangium is shown in figure 3, *a*. The swelling at the tip of the hypha is cut off by a cross-wall. An emptied cell is shown in figure 3, *b*. Others may undergo a resting period. These sporangia may remain terminal (following 3, *a-c-b*) or during the resting period a new hypha may be formed at the tip. It is interesting to note that only a portion of the protoplast of sporangium enters the young hypha which is now cut off by a new cross wall. The result is that the sporangium now becomes intercalary. This is shown in figure 3, $a$-$c$-$c_2$. The transition from $c$ to $c_2$ took place in $6\frac{1}{2}$ hours. The new upper hypha is less in diameter than the older portion and may continue to grow, to branch and to build new organs. The latter can be accomplished by a recapitulation of 3, *a-b* or 3, $a$-$c_2$ or it may occur as a possibility as arising from *d* in figure 3. The latter type may be more readily and more frequently observed.

First an intercalary swelling appears. Then, as shown in figure 3, *e* the upper cross wall is laid down, this differs only in minor details from the manner in which a terminal cell is formed, but

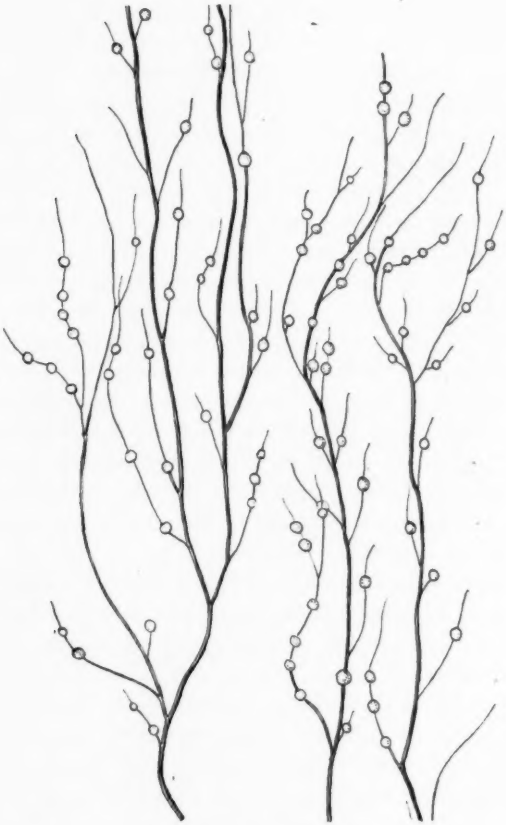


FIG. 2. Type of growth.

the intercalary cell is often oval in outline. There is a conspicuous movement of protoplasm from the part of the hypha below into this developing cell before the lower cross-wall is formed.

Both portions of the hypha, that above as well as that below the intercalary cell, may continue to develop other sporangia or to develop side branches. No branches were observed immediately

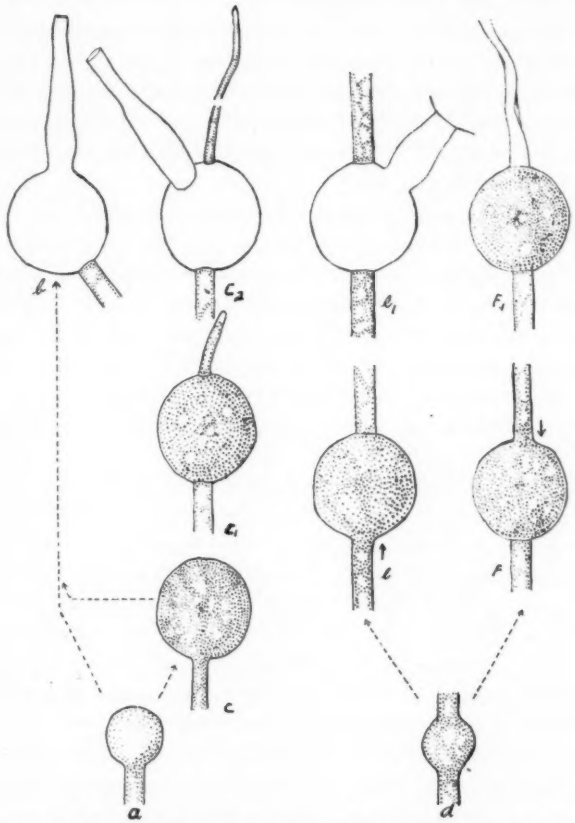



FIG. 3. Zoösporangium and resting-sporangium development. *b*, terminal sporangium. The development from *a–b* took at least 2 1/4 hours; *c–c*$_2$, a terminal sporangium becomes an intercalary one. This drawn example *c–c*$_2$ took from 10:40 A. M.—4:30 P. M.; *e–e*$_1$, an intercalary sporangium filled from below: *f–f*$_1$, an intercalary one filled from above.

below the intercalary sporangium, although it is possible that such hyphae may be formed. The second mode of development shown in figure 3, *d-f* is observed if the lower wall is first formed.

The tip portion now empties its contents into the developing sporangium before the upper cross-wall is formed. This type of behavior has been observed only in the upper part of a hypha, at the margin of the fungal weft.

These observations are not given in order to separate types but to explain the rarity of the terminal sporangia and to suggest that these modifications may account for the method of formation of the so-called "gemmae" whose behavior is the same as that of resting sporangia. The process of formation would be a modification of figure 3, *d-f*.

In older hyphae accumulation of protoplasts appeared. They varied greatly in their form as shown in figure 4, *a*, *b*, and *c*. These accumulations may take place in any portion of a hypha but they are more frequently found toward the tips. They are quite comparable to similar structures found among the Saprolegniaceae where they often occur in continuous rows. Here they are however most often found singly and not associated with oögones or sporangia. They are usually about 8–10 times the hypha diameter in length.

Butler (**1907**) with reference to Maurizio (**1894**), states that the gemmae among the Pythiaceae are not of the same value as those which are found among the Saprolegniaceae. The difference as stated by Maurizio,[3] is that the *Saprolegnia* gemmae are "sporangium anlagen," that is, they are a priori potential sporangia and later may germinate or sporulate. Butler denies this as the condition of the *Pythium* gemmae, "they are not of the same category." The following indicates the similarity of behavior of both resting-sporangia and gemmae.

The resting-sporangium of *Pythium* forms either zoöspores or germ-tubes. The older the resting-sporangium the less often does it produce zoöspores. After 6 weeks only rarely are zoöspores formed. A simple experiment readily illustrates this fact. To a turf of mycelium 6 weeks old which had been left undisturbed, an ant-larva was added. After two hours many

[3] Maurizio's paper of 1894, "Zur Entwicklungsgeschichte und Systematik der Saprolegnieen," Flora 109 pp. is meant, not that of 1896, "Die Sporangiumanlage der Gattung Saprolegnia," Jahrb. Wiss. Bot., p. 75 ff. and only so far as this single question is concerned. His terminology and the interpretation he gave (**1896**) cannot be accepted.

resting sporangia formed germ tubes (FIG. 4, *e*) but none formed zoöspores. The ant larva was removed and it was then noted that the germ-tubes formed new sporangia (FIG. 4, *d*). Some of these were placed in a water-mount in fresh filtered water for observation under the microscope. After 1 hour a few were already empty, in others zoöspores were being formed while by far the greater number remained as resting sporangia again. Now an ant larva was placed in the water mount at a distance of 1 cm. and in a short time germination occurred.

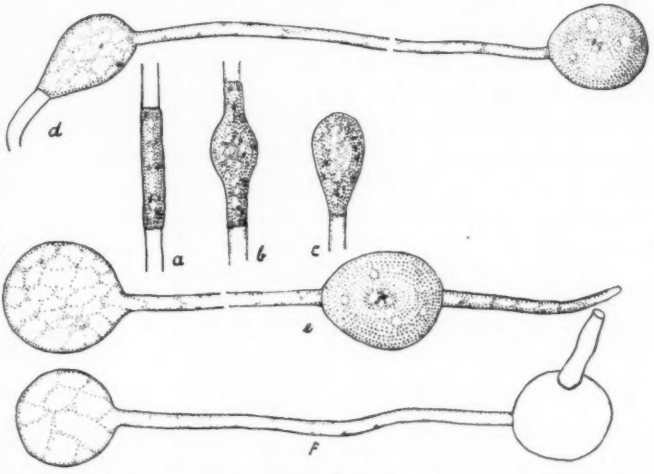



FIG. 4. *a*, *b*, and *c*, different forms of gemmae; *d*, germinating gemma produced a sporangium; *e*, an old germinating resting-sporangium produced a new intercalary resting-sporangium. *f*, an old resting-sporangium formed a sporulating sporangium.

This experiment was repeated for a further study of the behavior of the gemmae. They showed the same reactions. The old gemmae germinated and were able to form sporangia of regular shape, spherical or oval (FIG. 4, *d*). A few of these young sporangia developed from gemmae sporulated; the others became resting sporangia.

When the sporangia of regular forms were developed, thus using up the greatest part of the plasm, irregular plasm-accumulations appeared (like those shown in figure 4, *a*, *b*, and *c*) in

the longer germ-tubes of the resting-sporangia as well as in those of the gemmae.—At all times the sporangia, the resting sporangia and the gemmae showed the same behavior and therefore they can hardly be separated by means of different potential qualities.—Of interest is the question, whether the reduced ability of sporulation with increasing age is connected with cytological conditions.


FIG. 5. Ratio for intercalary and terminal oögonia.

In view of the terminology of the vegetative propagation-organs of this species it can be said that the term sporangium could include the immediately sporulating sporangium as well as the resting-sporangium and the gemma. The first two kinds are developed during the vegetative period of the mycelium and show a regular form, while the gemmae appear at the end of the growth in length of the hyphae or later on and are therefore of irregular form. This is the only difference which might have recommended that sporangia and gemmae be spoken of separately.

Formations for which the term "chlamydospore" is again proposed by Rosenbaum (**1915**) and Dissmann (**1927**) did not occur.

## SEXUAL REPRODUCTION

After 36 hours on ant-larvae, within a water-dish, sexual organs developed.

The formation of the oögone is very similar to that of an intercalary sporangium except that the diameter remains shorter. This formation is usually intercalary, very seldom terminal. Figure 5 [4] shows the ratio between the two types.

When the swelling has reached the final size of the oögonium, the adjoining parts of the hypha are densely filled with plasm. After a short time (10–30 minutes) swellings appear within the hypha and then cross-walls become visible, which separate parts of the hypha about 9–14 $\mu$ long and which will become the antheridia. In most cases 2 antheridia are present, one hypogynous and one epigynous. Sometimes only one is present and then it may be either hypogynous or epigynous. Figure 6 shows the ratio of the three possible types.


FIG 6. Ratio for the antheridia.

The plasm within the oögone does not completely fill the oögone. It has at first a smooth outline. Very soon (see the explanation of plate 20 on page 507) the plasm-surface becomes irregular and the plasm undergoes accumulations and translocations. The result is the separation of the periplasm which degenerates.

During this time one antheridium has dissolved the oögone-wall and a part of its plasm enters. Corresponding to that one or two vacuoles appear within the antheridium. When the oögone-plasm again has a smooth outline, a tube is distinctly visible arising from the antheridium and reaching the oögone-plasm. In the meantime the second antheridium has broken through the oögone-wall and forms a similar tube. Both antheridia present

[4] To take the values for the graphs figures 5, 6, and 7 the third, the fifth and sixth daughter-cultures were used. Those oögonia were taken which showed clearly the necessary facts and which were found during one continued examination.

become emptied; no exception could be found. A very small remainder is left in the antheridium which soon disappears.

Then the oögone-plasm seems to enlarge. After 70 minutes the outer line of the oöspore-membrane is visible; 1½ hours later also the inner line. This oöspore-membrane is thicker than the oögone-membrane and measures ± 1.5 μ. Slow translocations of the plasm continue.

The next day an irregularly outlined, bright-shining vacuole is surrounded by the oöspore-plasm (PLATE 20, FIG. 9) which is the first sign of the development of oil-drops. The next two days no progress could be seen. In an attempt to influence it, the window was left open over the cold night. After the second night in both oöspores continually observed the formation of the oil-drop was finished. They were smaller than the inner vacuole already present (PLATE 20, FIGS. 9 and 10).

The development of the oil-drops may take some weeks. After 9 weeks a number of oöspores can be found in which they are not yet finished. For a longer time a labile balance seems to exist and the possibilities are either oil-secretion or dissolution. However the formation of oil-drops is not absolutely necessary before germination takes place. Both kinds of oöspores have been seen germinating. My dates suggest that those oöspores without fully developed oil-drops germinate more readily for the dissolution of oil-drops is not necessary. But my notes also suggest that the oöspores with fully developed oil-drops are more resistant to environmental conditions.

Similar observations among Saprolegniaceae and *Allomyces* suggest furthermore, that the complete secretion can be accelerated by external factors. The brown resting-spores of *Allomyces* for example react to contact. If we carry them with a needle over on to agar, regularly one or a few oil-drops are secreted immediately. In other cases the temperature seems to be influential.

Either one or two oil-drops were found within the oöspores of *Pythium epigynum*. They are always surrounded by plasm and located slightly excentrically or close to the periphery. The outside structures of *Achlya oblongata* or *Pythiopsis cymosa* as drawn by Coker never occur.

The graphs in figure 7 show the diameters of the oöspores and oögones. The third, fifth and sixth daughter-cultures were used for these measurements.—Both oöspore- and oögone-curves were brought on one abzisse in order to have an illustration for the terms "oögone-filling" and "not filling." Ever since the establishment of the genus this has been a criterion and is used today. These two curves have a small common field *A-B-C*, which grows

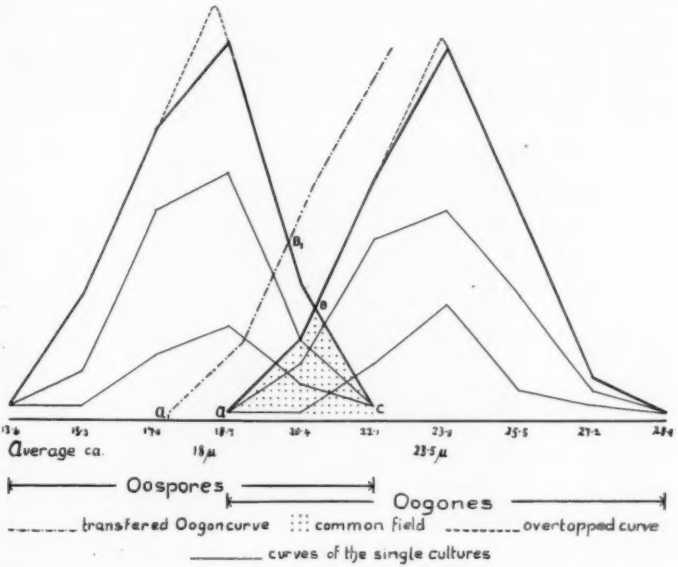



FIG. 7. Graph showing the average-diameter of oöspores and oögones. Explanation in the text.

slightly when the double thickness of the membrane is subtracted, which in this case is 1.5 μ. The common field is now $A_1$-$B_1$-$C$. It follows for *Pythium epigynum:* in most cases the oöspores do not fill the oögonia although some can be found which do fill the oögonium.

Two times an oögone was found each containing two oöspores. The latter were in these cases of different size and not spherical (PLATE 20, FIG. 12).

The germinating eggs observed formed a tube which branched. These hyphae are able to infect or to form sporangia, which have been shown to be of the same behavior as those described above.

## PARASITISM

When the above observations had been carried out the following experiments were made in order to see whether *Pythium epigynum* is a parasite.

For this purpose an apparatus used here in the physiology course was modified (FIG. 8).—The water from the faucet ran slowly but constantly into a suction-flask, which stood on a heater. The water temperature fluctuated between 70°–95° C.

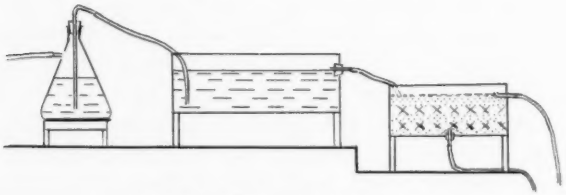


FIG. 8. Explanation in the text.

Thereby the possible fungus-spores within the water were killed. From here the heated water ran first into a covered water-container and then into a soil-case. The latter case contained soil, humus xxx and sand ˙ . ˙ . ˙ (both sterilized for several days within the autoclave) in several layers. An outlet kept for the first two days a water-layer 1 cm. in depth and then by the turning of the bent outlet the water-surface was lowered.

The average water-temperature within the water-container was about 24° C., while it was 19–23° C. within the soil-box. The latter temperature favors the seed-germination as well as fungus growth.

The seeds on the surface of the soil developed naturally. After a few days the radicula of peas and beans grew to a length of 0.4–0.9 cm. No disturbances were recognized. Then 4 wefts of *Pythium epigynum* on ant-larvae were brought into the water. After two to three days the germinating seeds were attacked and the fungus mycelium covered almost the whole soil-surface.

Those seeds which lay close to the ant-larvae died first. Those at a greater distance followed about 1 day later. Figure 9 gives a photograph of seeds being $4\frac{1}{2}$ days in water.

Grass-seed had been added at the same time. They germinated after 5 days. They developed naturally and seemed to be resistant. Later on, after 10–12 days, some collapsed. They were surrounded by a thick weft of the fungus but only one of the examined plants showed real infection. The hypocotyl was attacked. The hyphae coming from the plant formed a dense mass of vegetative propagation-organs. Here no sexual organs were found until the 14th day, while they have been found on peas and beans after 8–9 days.

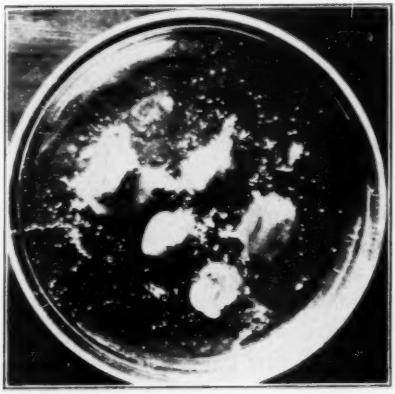


FIG. 9. Peas and beans affected by *P. epigynum*.

The parasitism of this species was confirmed by trials within flower-boxes. These boxes contained a mixture of 2/3 sand and 1/3 black humus, both parts having been sterilized several times. Grass seed, wheat, rye, corn, oats and barley were grown. After 15 days one fungus-weft was brought on the soil-surface of each box and kept moist by watering. Among these the corn-seedlings were attacked by the fungus. Discoloration of the plant appeared at the soil-surface. After 5 weeks the fungus was found within the root as well as on the stem and leaves.

Fungus-cultures, obtained from the affected seeds and seedlings proved *Pythium epigynum* to be a parasite.

## TAXONOMY

This oömycete belongs to the genus *Pythium* and furthermore to the sub-genus *Sphaerosporangium* (Fischer). It is characterized by the facts that only hypogynous and epigynous antheridia are present, that the oögones and oöspores differ in size and shape from related species and that production of so-called gemmae is found frequently. For the nearest related members of this group a scheme for comparison is given on page 505.

Epigynous antheridia have not been reported until recently. Matthews has figured for *Pythium pulchrum* v. Minden in plate 21, figure 4, 2 hypogynous antheridia. One of them might be an epigynous one. But she states the antheridia are hypogynous, androgynous or diclinous. Also von Minden (**1916**) might show on plate 6, figure 53 an epigynous one. However, because he states the antheridia are hypogynous, androgynous and diclinous, it might as well be a diclinous one since a similar figure of *Pythium Debaryanum* is shown, but it is stated as one hypogynous and one diclinous antheridium. In addition to that, *Pythium pulchrum* v. Minden differs distinctly in all other respects from *Pythium epigynum* (also the oögone and oöspore-measurements of Matthews are still larger than those of v. Minden).

A similarity seems to be present in *Pythium rostratum* Butler. But just the characteristics pronounced as important by Butler are different. He says of *Pythium rostratum* that the hyphae are "never prolonged into fine filaments" and "the oögonium is completely filled by the oöspore, . . . this is a character of specific value in the Pythiaceae. . . ." Furthermore compare both species on page 505 or Butler's text.—Confusion with *Pythium vexans* is scarcely possible.

On page 505 *Pythium Debaryanum* Hesse is not given. This species was also isolated and compared with *Pythium epigynum*. The typical growth under the same conditions and on ant-larvae is different. The hyphae of *P. Debaryanum* grow longer than 2 cm. and aërial hyphae are formed. Also the septation in old hyphae is much more common and appears in *P. Debaryanum* in the thick main-hyphae as well as within the branches. An empty neck is never found below the sporangium in *Pythium epigynum*. The "conidia" or resting-sporangia are smaller in

*P. Debaryanum* and the oögones are mostly terminal while in *Pythium epigynum* mostly intercalary (FIG. 5). Furthermore, a graph like that given in figure 7 drawn for *P. Debaryanum* would be strikingly different because the oöspores measure 14–18 $\mu$ and the oögones 20–25 $\mu$.

*P. artrotogus*, which forms also one hypogynous antheridium is in its appearance quite different.

Therefore this fungus is described as a new species.

| | P. pulchrum | P.epigynum | P.prostratum | P.vexans |
|---|---|---|---|---|
| Hyphae | diam 3-5 $\mu$ | diam 5-8 $\mu$ gradually getting thinner prolonged into fine filaments | diam 6-8 $\mu$ never prolonged into fine filaments | Branches are fine filaments |
| Sporangia |  terminal and intercalary, rows a clusters |  intercalary seldom terminal |  terminal seldom intercalary | Missing de Bary seldom Butler, term. or intercalary  |
| | spherical and oval | spherical and oval | spherical and ovoid | irregular pearshaped |
| | thin membrane collapsing when empty short tube | consistent membrane, long tubes, twice the sporangium-diameter | consistent membrane, tubes equal the sporangium-diam | short tubes |
| | |  Gemmae |  Gemmae rare seen once | "Canidia" |
| Oogonia | terminal and intercalary spherical 23-31 $\mu$ mostly 28 $\mu$ | intercalary, seldom terminal spherical 19-29 $\mu$ mostly 23.5 $\mu$ | intercalary and lateral transv. diam : 21 $\mu$ longit. " : more | lateral broad inserted 21-25 $\mu$ |
| Oospores | Oogone not filling 21-24 $\mu$, mostly 18 $\mu$ | Oogone not filling 14-22 $\mu$, mostly 18 $\mu$ | Oogone completely filling 12-26 $\mu$, mostly 21 $\mu$ | Oogone not filling 20-22 $\mu$ |
| Antheridia | hypogynous and androgynous, seldom diclinous | mostly 2, hypogynous and epigynous | single, androgynous, seldom hypogynous | 1, seldom 2, androgynous, seldom hypogynous |

**Pythium epigynum** sp. nov.

Fungus generat intra 2–3 dies circum nympham mycelium cum radio 1–15 cm. Hyphae singularis graciles et ramosae sunt et crassae 5–8 $\mu$. Rami attenuati sunt secundum ordinem. Apices filamentosa sunt.

Zoösporangia port 24 horas oriuntur, plerumque intercalaria, rarissime terminalia. Forma eorum globosa aut ovota est. Sporangia globosa diametros 29 $\mu$ (21–34 $\mu$) habent; ovata 19–24 $\mu$ longa sunt et 22–29 $\mu$ crassa.—Plasma per tubulum longum exit et ante orificium sporangii in vesicula manet. Nunc zoösporae oriuntur quarum utraeque cilias duas lateralis et vacuolam unam contractilem habent. Formatae, versicalem dirumpunt et per 10–30 min. emanant. Deinde requiescunt, rotundatae mem-

branam formant. Singulariter bis emanant. Tum germinant.—His sporangiis primis sporangia perdurantia forma et magnitudine fere similia sunt. Evacuatio eorum aut fit aeque illis aut plerumque germinant. Si nutrimentum adest utriculi inficiunt aut in aqua recente zoösporangia producunt sive rursum sporangia perduratia.

Organa sexualia iam post 36 horas oriuntur. Oögonia plerumque intercalaria, rarius terminalia sunt. Diametros eorum 19–29 $\mu$, in media magnitudine 23.5 $\mu$.

Antheridia semper adsunt, plerumque dua, et hypogynum et epigynum. Utrumque antheridium in oögonium evacuatur. Rarius antheridium unum solum oritur; aut hypogynum aut epigynum. Longitudo eorum 18 $\mu$ est.

In hyphis veteribus plasma in partes breves contracta septis transversalibus separatur. Gemmae nominantur; cum utriculo germinant qui inficere potest et zoösporangia aut sporangia perdurantia producere.

Fungus ex particula soli separatus est quae ex ripa lacus pratensis apud Milton, Wisconsin excepta erat. Fundus luto sabulosa constans sine vita plantae erat, campus graminosus 3 m. distat.

I am indebted to Dr. E. M. Gilbert for his advice and assistance during the preparation of this paper.

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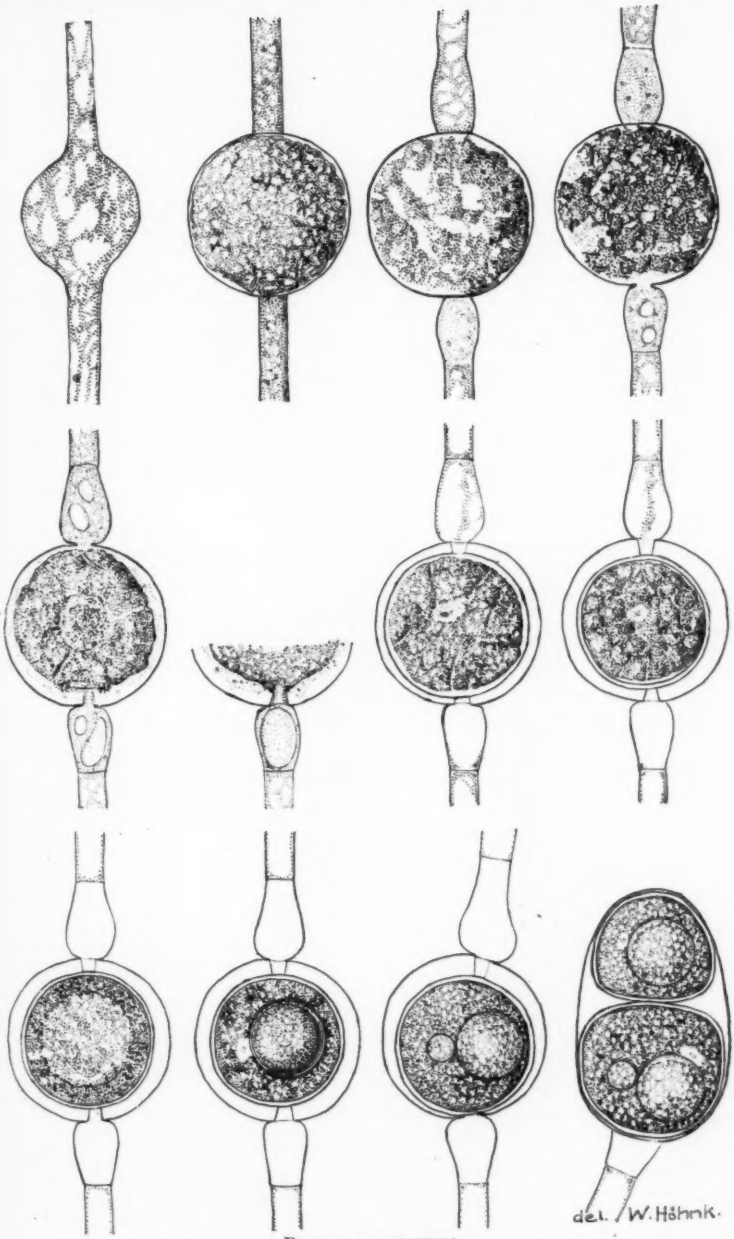


PYTHIUM EPIGYNUM

## EXPLANATION OF PLATE 20

Figs. 1–10 are taken from the same oögone under continued observation:

Oct. 24, 1931

1, 10:14 A.M. young, not yet separated swelling; 2, 1:40 P.M. crosswalls are formed; 3, 2:25 P.M. the oögone plasma has become irregular. Between stripes and balls of thick plasm bright, hyaline spaces are visible. The hypogynous antheridium is separated, the epigynous one is going to be formed; 4, 3:25 P.M. the oögone-plasma is at the highest point of action. The periplasm seems to be already formed. The hypogynous antheridium penetrates the oögone-membrane, the epigynous one shows the cross-wall; 5, 4:02 P.M. the oögone-plasma is differentiated. The inner becoming oöspore-plasm is surrounded by the periplasm. The hypogynous antheridium forms the tube to the egg plasm and the growth of the vacuole suggests the entering of the plasm; 6, the mouth-like swelling of the oöspore-plasm and differentiation within the periplasm. 7:05 P.M. the oöspore-plasm is surrounded by a bright shining circle; the outer wall of the oöspore-membrane is visible, which cuts off the antheridial tubes. The content of both antheridia has entered into the oöspore, and only a small remainder is left. Near the center a refractive vacuole.

Oct. 25, 1931

8, 1:00 A.M. the oöspore-membrane is ready. The plasm is becoming more and more homogeneous.

Oct. 26, 1931

9, 10:05 A.M. the plasm secretes reserve material into the inner space. (The bright shining vacuole wandered to the left side.)

Oct. 28, 1931

10, 8:12 A.M. the central oil-drop is formed. The adjoining parts of the hypha are empty.

11, One oöspore with two oildrops; 12, One oögonium with 2 öospores of different size.

# THE GENUS PROTODONTIA

G. W. MARTIN

(WITH 2 TEXT FIGURES)

In 1895, Möller described the genus *Protohydnum* [1] from Brazil, with the single species *P. cartilagineum*, described as resupinate, yellowish white, and thickly beset with blunt spines attaining a length of 5 mm. This description is confirmed by an accompanying photographic illustration (PLATE 3, FIG. 1). The basidia are divided longitudinally into four cells, each cell producing an epibasidium tipped by a sterigma and bearing an elongated, oval spore 9 × 4–5 μ. In 1903 Bresadola doubtfully attributed to this genus a second species, found in Poland, as *?Protohydnum lividum*.[2] This species was clearly distinct from Möller's, being livid fuscous to vinaceous fuscous, waxy membranous in texture and with small, acute spines about 0.5 mm. in length, with sterile tips, the spores subglobose to obovate, depressed on one side and with a large guttule, 5.5–8 × 4–5 μ.

In 1907 von Höhnel described the genus *Protodontia* [3] to accommodate a fungus with the aspect of *Odontia* but characterized by longitudinally septate basidia. This species, named *P. uda*, was described as yellowish or reddish yellow, with short spines 0.2–0.4 mm. long, and with broadly ovate spores, flattened on one side, 6–8 × 4–5 μ. Rea [4] reports this species as rare in Britain, using von Höhnel's name, but noting that it is pure white when fresh, and giving the spore measurements as 6–8 (–9) × 3–4 μ. Bourdot and Galzin,[5] on the other hand, regard this species as identical with that of Bresadola, use his name, and report its occurrence in France. They describe the color as "grayish hyaline, a trifle bluish," the spines livid-fawn when dry, the spores ovoid, somewhat depressed laterally, 5–9 × 4–6 μ. They also describe a form *microdon* with slender, short spines

[1] Protobasidiomyceten 131. 1895.
[2] Ann. Myc. **1**: 117.
[3] Sitzungsber. Acad. Wien. **116**: 83.
[4] British Basidiomycetae 736. 1922.
[5] Hymenomycètes de France **1**: 34. 1927.

and spores 6–7 × 3–4 μ and a var. *furfuracea* from France with white, furfuraceous-pulverulent subiculum and spores 5–7 × 3–4 μ.

It will be observed that there is some confusion between these various descriptions. It seems clear, however, that *Protodontia* is sufficiently distinct from *Protohydnum* to be maintained as a valid genus. Whether von Höhnel's species is the same as the forms described by Rea using von Höhnel's name is not certain, but seems probable, as slight variation in spore size and color is not of great significance in the Tremellales. That Bresadola's species is the same is very questionable, the differences in color and habit being too great. It may not belong to either *Protohydnum* or *Protodontia*. The description of *Protohydnum lividum* Bres. and its variants, as given in Bourdot and Galzin, is rather broad, but would seem to refer to von Höhnel's species and not to Bresadola's.

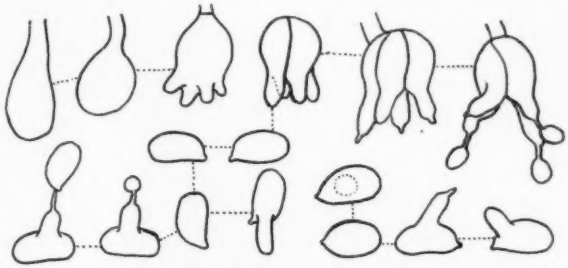

FIG. 1. Basidia and spores of *Protodontia uda* v. Höhn. Above, six basidia in successive stages of development; below, ten spores, five in various stages of germination, × 1500. The dotted lines indicate that the figures so connected are from the same fructification.

We have several collections from Iowa of a fungus with the aspect of *Odontia*, but of subgelatinous consistency and bearing in its hymenium typical tremellaceous basidia. The fructification is resupinate, with indeterminate margins, the subiculum waxy, very thin, sometimes continuous but commonly broken or reticulate, especially near the margins. The spines are white, waxy, slender, approximately terete, 0.1 to 1 mm. in length, occasionally longer, and usually more or less branched, appearing fimbriate under a lens (FIG. 2); the larger spines are often sterile,

evidently because the basidia have discharged their spores and collapsed. The basidia are clavate, the swollen part nearly globose, 12–14 × 5–6 μ, becoming longitudinally septate, each of the four divisions producing a rather short epibasidium bearing a spore in the usual fashion (FIG. 1). The spores are oval or short cylindric, slightly curved, 5–7.5 × 2.5–4 μ, germinating by repetition. The fructifications are watery white or grayish white when fresh, drying yellowish. They are usually small, 1–3 cm. broad, but one collection was over 10 cm. long and half as wide.

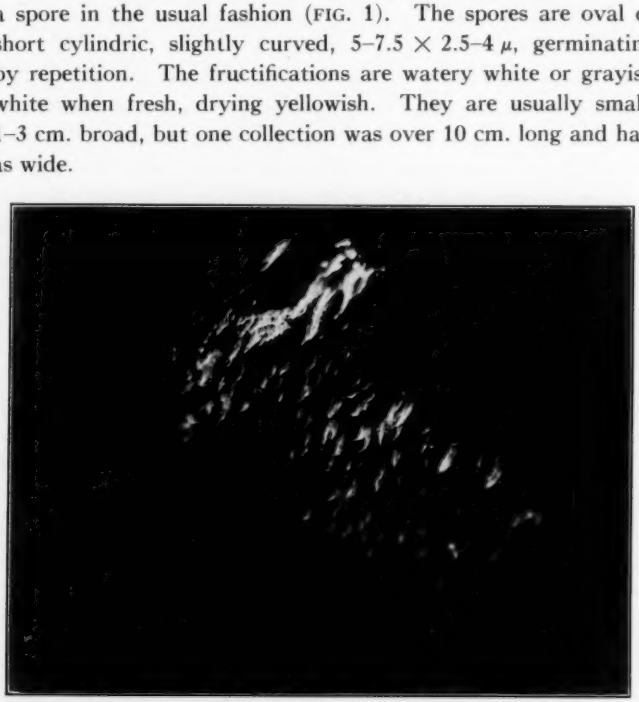

FIG. 2. Hymenium of *Protodontia uda*, showing character of spines. About × 15.

In spite of the somewhat smaller spores I am inclined to believe our species is merely a local variation of *Protodontia uda* von Höhnel, possibly nearer the var. *furfuracea* than to the typical form. The resemblance to *Odontia* is superficial, as the branches of the spines are of the same structure as the main axis, being composed of nearly parallel hyphae which are terminated by the basidia borne along the sides. There is no trace of cystidia.

It seems clear that *Protohydnum* should be retained for forms with a distinct context and blunt, finger-like spines, while species

with acute, fimbriate spines borne on a scanty subiculum are better referred to *Protodontia*. The species described and illustrated by Albertini and Schweinitz as *Hydnum fasciculare* [6] was transferred by Fries to *Mucronella* and by Bresadola [7] to *Protohydnum*, in which genus it is retained by Bourdot and Galzin. It is certainly distinct from *Protodontia uda* and is not a *Protohydnum* in Möller's sense as that is here interpreted. The *Mucronella*-like clusters of spines, connected merely by what Bresadola calls a pseudo-subiculum, may even justify its generic separation from *Protodontia*. Killermann [8] recognizes both genera, but his diagnoses are brief and of little help. He includes only the one species in *Protodontia*, *P. uda*, while in *Protohydnum* he includes with Möller's original species, *P. cartilagineum*, both *P. lividum* and *P. fasciculare*.

A collection in the herbarium of the Missouri Botanical Garden (M. B. G. *63156*), determined by Burt as *Protohydnum lividum* Bres. was collected by Langlois in Louisiana and labelled by him "*Exidia glandulosa?*". It has a well developed, separable context bearing a continuous hymenium, with a very few widely scattered, minute teeth. In color it is vinaceous livid when moist, drying to a thin, horny, reddish crust. The spores are elongate, slightly curved, 12–14 × 5.5 μ. Except for the few spines, it would be a typical *Sebacina*. It certainly does not agree with Bresadola's description.

The Iowa collections are from three distinct localities, one, in Dubuque County, separated by over a hundred miles from the other two, in Johnson County. In addition we have a portion of a collection from Louisiana, gathered by Mr. Clair A. Brown (No. *344*) at Sorrento, which is apparently the same species, athough the teeth are somewhat less fimbriate and there are insignificant differences in spore dimensions. It seems probable, therefore, that *Protodontia uda* is widely distributed and not uncommon, but is passed over by collectors as a poorly developed or weathered *Odontia*.

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[6] Consp. Fung. 269. 1805.
[7] Ann. Myc. **18**: 63. 1920.
[8] In E. and P. Nat.-Pfl. 2 ed. **6**: 118–119. 1928.

# THE VIABILITY OF CULTURES OF RHIZOPUS NIGRICANS

EVELYN I. CARPENTER

From time to time, various investigators have given data, either directly or indirectly, which provide some information concerning a possible time period during which cultures or spores of fungi may retain their viability. Povah (**2**) has shown that cultures of fungi which fail to grow when transferred to fresh agar slants may do so when given a certain hot agar treatment. This proves that the longevity of a culture is not necessarily correlated with the success or failure to obtain a subculture by means of a simple transfer of spores or mycelium, and therefore the latter condition should not be used as a criterion of the lack of viability.

It was while procuring information as to the longevity of certain cultures that the results here presented became evident. These the writer wishes to contribute as her mite to the gradual accumulation of data.

On December 16, 1926, Professor Don M. Benedict, College of Forestry, Syracuse University, received from Dr. A. F. Blakeslee cultures of the plus and minus strains of *Rhizopus nigricans* Ehr. to replace those which were supposedly lost. These cultures consisted of mycelium and spores folded in the customary sterile paper packets. Since it became unnecessary to use the cultures, they were filed away with Dr. Blakeslee's letter inclosed. On March 2, 1932, these packets, still unopened, were turned over to the writer, who transferred the contents to sterile Blakeslee's dextrose-malt-agar slants. The material comprising the plus strain was distributed between two such slants, while only one transfer could be made of the minus strain. Within four days both tubes containing the plus strain showed the characteristic growth of *Rhizopus nigricans* Ehr. The tube to which the minus strain had been transferred failed to show the presence of any growth.

Since, in making the sub-cultures, both mycelium and spores were transferred, there may well arise the question, whether growth resulted from the spores or the mycelium or both. In view of this fact, the packet in which the plus strain had been kept was carefully re-examined, and an abundance of free spores was found. These spores were transferred to sterile slants, especial care being taken to transfer no mycelium. After eight days there were no signs of growth. Benedict, in an unpublished paper, suggests a modification of Povah's hot agar method. Sterile distilled water, boiled for three minutes and allowed to cool for five minutes, was found to revive old cultures of *Coprinus sterquilinus* Fries. Therefore, a small amount of sterile distilled water, which had been previously heated to the boiling point and cooled for four minutes, was poured over the spores on the slants. A check was made to prove that water at this temperature does not kill viable spores. Three days later, there was still no evidence of growth. Povah's hot agar treatment likewise failed to stimulate the spores to germinate. Miss Ferguson (1) working with *Agaricus campestris* (L.) Fries, found that germination of spores was best and most constant when living strands of the mycelium of the same fungus were present. In order to indicate that any growth which might occur would be from spores only, mycelium, killed by heating, was added to one of the above slants. Still no growth resulted. While spores have long been considered the means by which unfavorable periods are bridged, still it is equally certain that fungous mycelium has a greater resistance to unfavorable conditions than is commonly supposed or accepted. The writer hopes in the future to offer precise data in support of this assertion.

The resumption of growth by the plus culture used in this experiment occurred after a time interval of five years, two months and three days. This growth resulted from a transfer in the usual fashion, without any stimulation other than a medium at normal room temperature. The longest time interval recorded by Povah (loc. cit.) for cultures of *Rhizopus nigricans* Ehr. is less than one year (eleven months and six days). The writer believes that many mycologists could record periods of over one year during which cultures of *Rhizopus nigricans* Ehr. have remained

viable. The query therefore arises whether it was necessary for Povah to use his hot agar treatment in order to revive his culture. For, if cultures of this fungus, of less than one year, will not produce viable transfers without the hot agar treatment, then they must have been subjected to severe desiccation or other extreme factors.

Within the writer's knowledge there is no recorded time interval for a single culture of *Rhizopus nigricans* Ehr. longer than the one found in this experiment. It is therefore believed that the results obtained impart important mycological information.

This discussion leads to the following conclusions:

1. Data concerning the longevity of cultures, spores or mycelium, based upon simple transfers are not reliable. This is indicated in Povah's paper and further proof is evinced in the experiment here stated.

2. This experiment does not prove that the spores did not germinate in the presence of the mycelium, but no germination of the spores resulted in its absence.

3. The mycelium definitely retained its viability for the time interval of five years, two months and three days, and is the important factor in the longevity of *cultures* of *Rhizopus nigricans* Ehr.

The writer is indebted to Mr. Benedict for the material used in this investigation, and for the suggestion which has resulted in its completion.

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## LITERATURE CITED

1. **Ferguson, M. C.** Germination of the spores of *Agaricus campestris* and other basidiomycetous fungi. Bur. Plant Ind. U. S. Dept. Agr. Bull. 16. 1902.

2. **Povah, A. H. W.** Notes upon reviving old cultures. Mycologia, **19**: 317. 1927.

## NOTES AND BRIEF ARTICLES

Dr. J. C. Arthur is preparing a "Manual of the Rusts of the United States and Canada," and will be pleased to receive specimens which add to the information in the seventh volume of the North American Flora. The new work will depart materially from the treatment in the N. Amer. Flora, in a way to render it more generally serviceable, and also to include illustrations.

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### The Mycological Society of America

At the last winter meeting of the Botanical Society of America, held at New Orleans, the Mycological Section formed a new society, The Mycological Society of America. The officers of the new organization are president W. H. Weston, jr., secretary-treasurer H. M. Fitzpatrick, and councilors H. S. Jackson, C. R. Orton, and Neil E. Stevens. The Society will hold its first meeting at Atlantic City, December 28–30, in affiliation with the American Association for the Advancement of Science.

As the new Society was formed near the end of the New Orleans meeting, and many who were interested were not present, it is hoped that there will be a full attendance at Atlantic City. A business session will be held Wednesday morning, December 28, at which it is expected that action will be taken on the proposition of the adoption of MYCOLOGIA as the official organ of the Society. A tentative contract has been drawn up between the officers of the Society and the New York Botanical Garden, which will be offered to the Society for its approval. By the terms of the contract the journal will continue to be published by the New York Botanical Garden, but the editorial policies will be controlled by the Society.

Membership application blanks are being mailed to all members of the former Mycological Section of the Botanical Society of America, to all personal subscribers to MYCOLOGIA, and to other selected lists of names. All persons in America and abroad who are interested in mycology in any of its phases are invited to make application for charter membership. It is expected that

the annual dues will not exceed five dollars, but this will be decided by vote of the Society.

At Atlantic City in addition to consideration of business matters there will be a scientific program, with several sessions for the reading of papers. Those who wish to present papers will be provided on request with a blank form which must be filled in and returned not later than November 3 to the secretary-treasurer.

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# INDEX TO VOLUME XXIV

New names and the final members of new combinations are in **bold face type.** Mistakes in the spelling of scientific names encountered in text are corrected in the index.